

New altitudinal record of *Myotis moratellii* Novaes et al., 2021 (Chiroptera, Vespertilionidae) in the northwestern foothills of Ecuador

Francisco X. Castellanos^{1,2}, Laura Simba^{2,3}, Jorge Brito²

¹ Department of Biological Sciences, Texas Tech University, Lubbock, Texas, United States of America

² División de Mastozoología, Instituto Nacional de Biodiversidad (INABIO), Quito, Pichincha, Ecuador

³ Facultad de Ciencias Biológicas, Universidad Central del Ecuador, Quito, Pichincha, Ecuador

Corresponding author: Francisco X. Castellanos (xacfran@gmail.com)

Abstract. *Myotis moratellii* Novaes et al., 2021 is a bat species that inhabits western Ecuador at low elevations (0–150 m a.s.l.). This study extends its northern distribution by 63 km into the foothill rainforests of the Chocó in Imbabura Province at 1,240 m a.s.l. Given the novel color patterns observed in the specimens collected, molecular approaches were necessary for species-level identification. Although the cause of this variation remains unclear, continued integrative taxonomy is critical to uncover the cryptic diversity within the genus.

Key words. Chocó, color variation, cryptic diversity, cytochrome b, distribution, elevation, premontane forest

Castellanos FX, Simba L, Brito J (2025) New altitudinal record of *Myotis moratellii* Novaes et al., 2021 (Chiroptera, Vespertilionidae) in the northwestern foothills of Ecuador. *Check List* 21 (3): 506–514. <https://doi.org/10.15560/21.3.506>

INTRODUCTION

Bats are one of Ecuador's most studied orders of mammals (see Albuja 1982, 1999; Albuja and Mena 2004; Larsen et al. 2012; Tirira 2017; Carrión-Bonilla et al. 2024). To date, 182 species of bats have been recorded in the country (Tirira et al. 2024). The knowledge of bat diversity in Ecuador has improved recently through taxonomic revisions integrating morphological and molecular data to identify previously unrecognized species within cryptic complexes (see Novaes et al. 2021; Yáñez-Fernández et al. 2023; Camacho et al. 2024).

This is particularly evident in *Myotis* Kaup, 1829, the most diverse genus of bats comprising 139 recognized species in the world (Simmons and Cirranello 2025). In Ecuador, 10 species have been reported (Carrión-Bonilla and Cook 2020; Novaes et al. 2021; Carrión-Bonilla et al. 2024), including cryptic species complexes such as *M. riparius* Handley, 1960 (Novaes et al. 2024) and *M. keaysi* J.A. Allen, 1914 (Novaes et al. 2021). In the last decade, increased surveys efforts in museums and surveys in previously unexplored geographic regions (Moratelli et al. 2011, 2013; Moratelli and Wilson 2015; Carrión-Bonilla et al. 2024), coupled with integrative taxonomy (Novaes et al. 2024) and the analysis of Ultraconserved Elements (Korstian et al. 2024), have shed light on the evolutionary relationships of this rapid radiation of bats.

These efforts have led to the description of new species, including one from the *M. keaysi* complex inhabiting the western coast of Ecuador (Novaes et al. 2021). *Myotis moratellii* Novaes et al., 2021 is a medium-sized species in the ruber group (Novaes et al. 2021), which has records from the provinces of El Oro, Los Ríos, and Esmeraldas, yet its habitat has been restricted to the tropical monsoon ecoregion in seasonal dry forests of the Chocó and tropical broadleaf dry forests at maximum altitudes of 150 m a.s.l. (Novaes et al. 2021). However, these geographic and ecological boundaries are sometimes an artifact of limited sampling of poorly known species as demonstrated for other Neotropical *Myotis* by Carrión-Bonilla et al. (2024). The northwestern region in Ecuador, for example, is typically overlooked due to its challenging topographic and logistical complexities (Brito et al. 2020). As a result, local species composition and genetic variation remain unknown and further complicated by cryptic species complexes for which species-level identification via morphologic analysis can be unreliable (Korstian et al. 2024).

Here, we document a new altitudinal record for *M. moratellii* in the Reserva Río Manduriacu, which also extends its northern distribution in Ecuador.



Academic editor: Héctor Ramírez-Chaves

Received: 18 February 2025

Accepted: 21 April 2025

Published: 9 May 2025

Copyright © The authors. This is an open-access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0)

METHODS

Collection and study of specimens. One sampling campaign was carried out in May 2024 in the Reserva Río Manduriacu in the Chocó ecoregion, in northwestern Ecuador. Sampling was carried out for 6 nights, using 8 mist nets of 6, 10 and 12 m (Kurta and Kunz 1988). The nets remained open between 18:30 and 21:30 pm with a total effort of 1,296 m/18 h (Medellin 1993). Standard external body measurements (mm) were taken post-mortem from each individual (total length, tail length, right forearm, right ear length, right tragus, weight (g)), as well as the sex and reproductive status. Cranial measurements of collected specimens were taken with digital calipers of precision of 0.1 mm. Body and cranial measurements, as well as other species-specific characteristics follow Novaes et al. (2021). To make fur color comparable to other studies, we followed the standardized color nomenclature of Ridgway (1912). Voucher specimens were deposited in the Instituto Nacional de Biodiversidad (INABIO), formerly, Museo Ecuatoriano de Ciencias Naturales (MECN), Quito, Ecuador. We preserved MECN 8004 as skin, skull, and skeleton, and MECN 8096 in 10% formalin.

The study was conducted under collection permit no. MAATE-ARSFC-2023-0145, and authorization of access to genetic resources no. MAATE-DBI-CM-2023-0334, both issued by the Ministerio del Ambiente, Agua y Transición Ecológica de Ecuador. Collecting of the specimens was made following the ethical guidelines of the American Society of Mammalogists (Sikes et al. 2016).

DNA extraction, sequence generation and assembly. To obtain sequence data, DNA was isolated from the liver using the GeneJET Genomic DNA Purification Kit (K0722). The amplification process of the full-length mitochondrial cytochrome b (Cytb) gene was achieved via Polymerase Chain Reaction (PCR) with the forward and reverse primers MVZ05 and MVZ16 (Smith and Patton 1993), and the GoTaq® Green Master Mix 2X kit. The PCR protocol included an initial denaturation step at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 45 °C for 30 s, and extension at 72 °C for 80 s, concluding with a final extension at 72 °C for 5 min, modified from Hurtado and Pacheco (2017). Sequencing of the Cytb was conducted using a MinION mk1c equipped with Flongle Flow Cells R10.4.1 and the Rapid Barcoding Kit 96 (SQK-RBK114.96), adhering to standard protocols. Data were basecalled with Dorado v. 0.5.2 and the high accuracy model v. 4.3.0., to obtain good quality sequences for phylogenetic analysis (Sanderson et al. 2024). The resulting fastq files were filtered at a Q score of 9, and consensus sequences were generated using NGSpecies ID v. 0.3.0 (Sahlin et al. 2021). DNA extraction, PCR amplification and sequencing with Oxford Nanopore Technologies were performed at the Nucleic Acid Sequencing Laboratory of the INABIO in Quito, Ecuador.

Phylogenetics analyses and genetic distances. To provide phylogenetic context, we downloaded Cytb sequences from NCBI for all species described to date in the ruber group (Carrión-Bonilla and Cook 2020; Novaes et al. 2021; Carrión-Bonilla et al. 2024), and three species from the albescens group as the out-group (Table 1A). The multiple sequence alignment (MSA) of 69 Cytb sequences was generated with the online MAFFT tool v. 7 with default settings, and resulted in an alignment containing 48 constant sites, 297 parsimony informative sites, and 374 distinct site patterns.

To assess assumptions of homogeneity and stationarity we tested three maximal matched-pair tests of symmetry (Naser-Khdour et al. 2019) in the MSA prior to phylogenetic analysis in IQ-TREE 2 v. 2.3.0 (Minh et al. 2020). Subsequently, the nucleotide substitution model for codon positions was estimated using ModelFinder (Kalyaanamoorthy et al. 2017) and selected via the Bayesian Information Criterion within the IQ-TREE 2 pipeline. The reconstruction of phylogenetic relationships using Maximum Likelihood (ML) was conducted with the best-fit Muse-Gaut model (Muse and Gaut 1994) with unequal nucleotide frequencies and four gamma categories (MG+F3X4+G4), selecting *Myotis levis* I. Geoffroy Saint-Hilaire, 1824 as the out-group taxon to root the tree. Nodal supports were calculated with the UltraFast Bootstrap approximation (UF; Minh et al. 2013) to reduce the impact of severe model violations, and the SH-approximate likelihood ratio test (SH; Guindon et al. 2010) with 1,000 bootstrap replicates.

To validate species identities, we employed the phylogenetic species concept (De Queiroz 2007) and considered a highly supported clade (UF \geq 95 and SH \geq 80, Minh et al. 2013) to represent a single evolutionary unit. Additionally, we used the genetic species concept as a predictor of species limits (Baker and Bradley 2006). We set a threshold of 3% as a measure of intraspecific genetic variation, and greater than 3% to delimit sister taxa as empirically seen for *Myotis* (see Baker and Bradley 2006 and references therein). To model genetic distances, we used MEGA v. 11.0.13 (Tamura et al. 2021). We first calculated the gamma parameter with the ML Estimate Rate Variation among Sites tool with four discrete categories and a moderate branch swap filter and then computed within and between-group divergence values with the Kimura 2-parameter model (Kimura 1981).

RESULTS

***Myotis moratellii* Novaes, Cláudio, Carrión-Bonilla, Abreu, Wilson, Maldonado & Weksler, 2021**
Figures 1–3

New record. ECUADOR — IMBABURA · Cotacachi, García Moreno, Reserva Río Manduriacu; 00.3104, –078.8578; 1,240 m alt.; 18.V.2024; F.X. Castellanos, L. Simba and J. Brito leg.; GenBank PV106025 and PV106026; 2 ♂, MECN 8004 (JBM 3257) and MECN 8096 (JBM 3279).

Identification. According to Novaes et al. (2021), *M. moratellii* is of medium size (forearm length 35–38.2 mm; total length 76–89 mm; greatest length of skull 13.79–14.05 mm). However, the specimens from Imbabura have slightly larger forearms but similar total length (Table 1). Ears are comparatively short with elongated tragus and the tip rounded (Figure 1).

The pelage of *M. moratellii* is woolly and moderately long with Buckthorn Brown unicolored dorsal hairs as in MECN 8004 (Figures 1A, 2); upper dorsum of uropatagium covered with Dresden Brown hair extending to just beyond the knees; plagiopatagium attached to the foot by a broad band of membrane (Figure 2), and an absent fringe of hairs on the distal border of the uropatagium. Central part of the abdomen Mummy Brown, with Ochraceous-Orange sides (Figure 2B). Conversely the ventral fur of MECN 8096 is Mummy Brown with a ventral and pectoral patch of Maize Yellow and Ochraceous-Orange hairs; the dorsal fur is Dresden Brown with lighter dispersed patches of Ochraceous-Orange hairs from the base of the tail towards the skull and rostrum (Figure 1B).

The skull of MECN 8004 is similar to the holotype (Table 1). Medium sagittal crest present; skull robust and broad; braincase inflated and noticeably high in profile; roof of the braincase formed by the parietal bone subtly sloping forward; frontal bone steeply sloping in lateral view; posterior region of the braincase rounded and projecting beyond the boundary of the occipital condyles; mastoid processes well developed (Figure 3).

Molecular analysis. The ML phylogenetic tree shows MECN 8004 and MECN 8096 nested in a highly supported clade with representatives of *M. moratellii* from the provinces of El Oro and Los Ríos (Figure 4A;

Figure 1. Fronto-lateral view of two live *Myotis moratellii* captured in the Reserva Río Manduriacu. **A.** MECN 8004. **B.** MECN 8096. Photos by Jorge Brito.

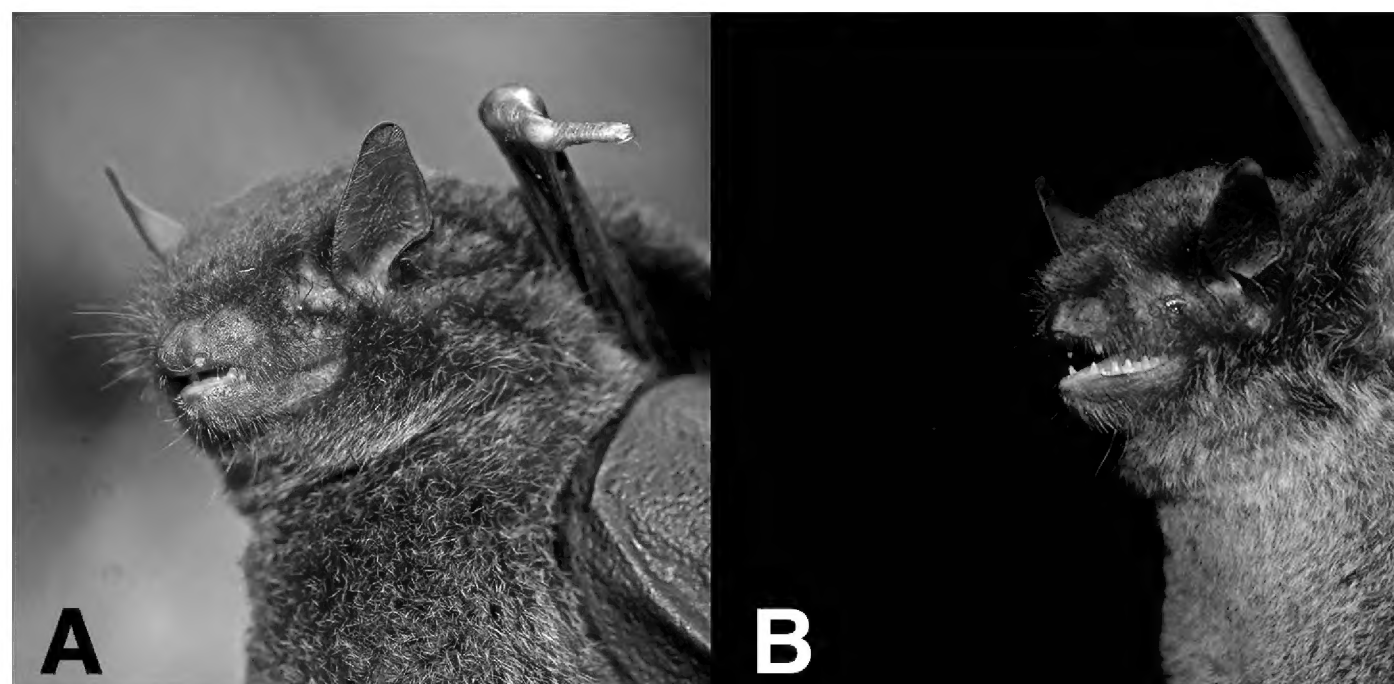


Figure 2. Whole body view of *Myotis moratellii* (MECN 8004) post-mortem. **A.** Dorsal view. **B.** Ventral view. Note Ochraceous-Orange patches of fur in both views. Scale bar = 5 mm. Photos by Jorge Brito.

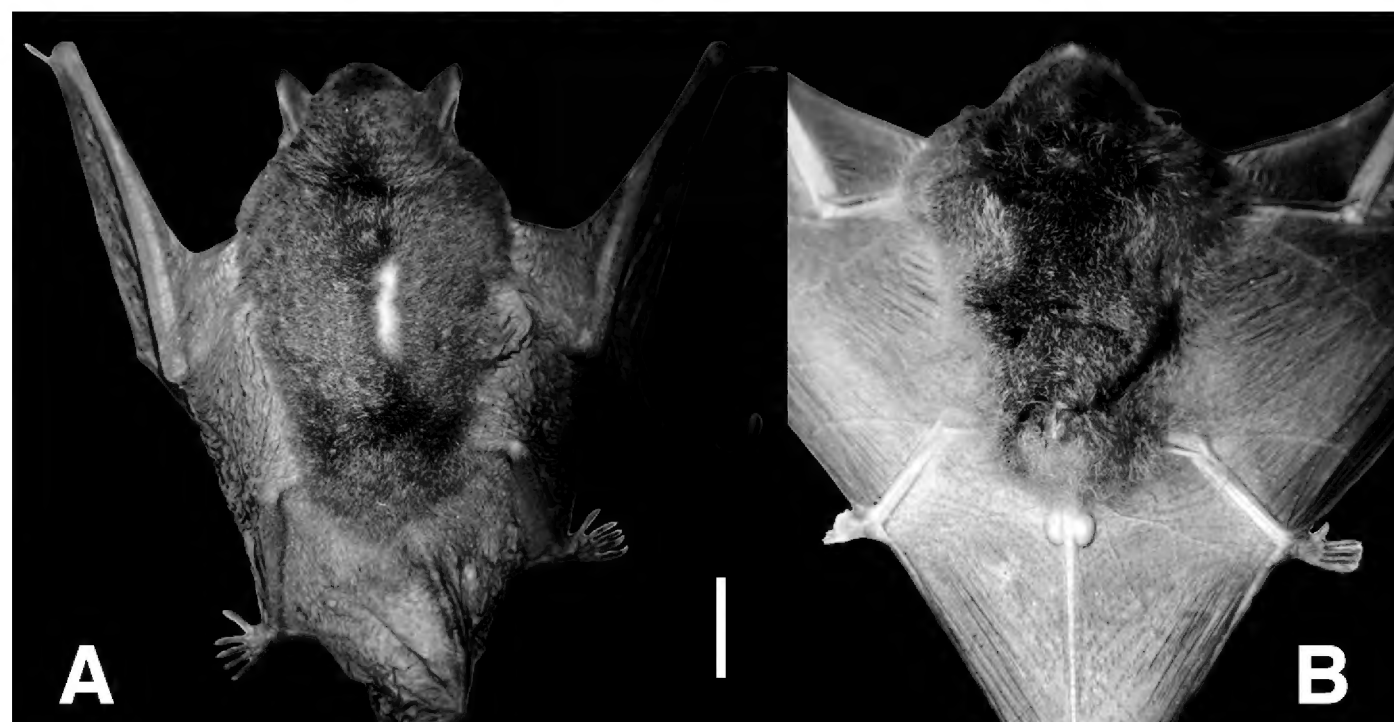


Figure 3. Dorsal, ventral, and lateral views of the skull, and lateral view of the mandible of *Myotis moratellii* (MECN 8004). Scale bar = 5 mm. Photos by Jorge Brito.

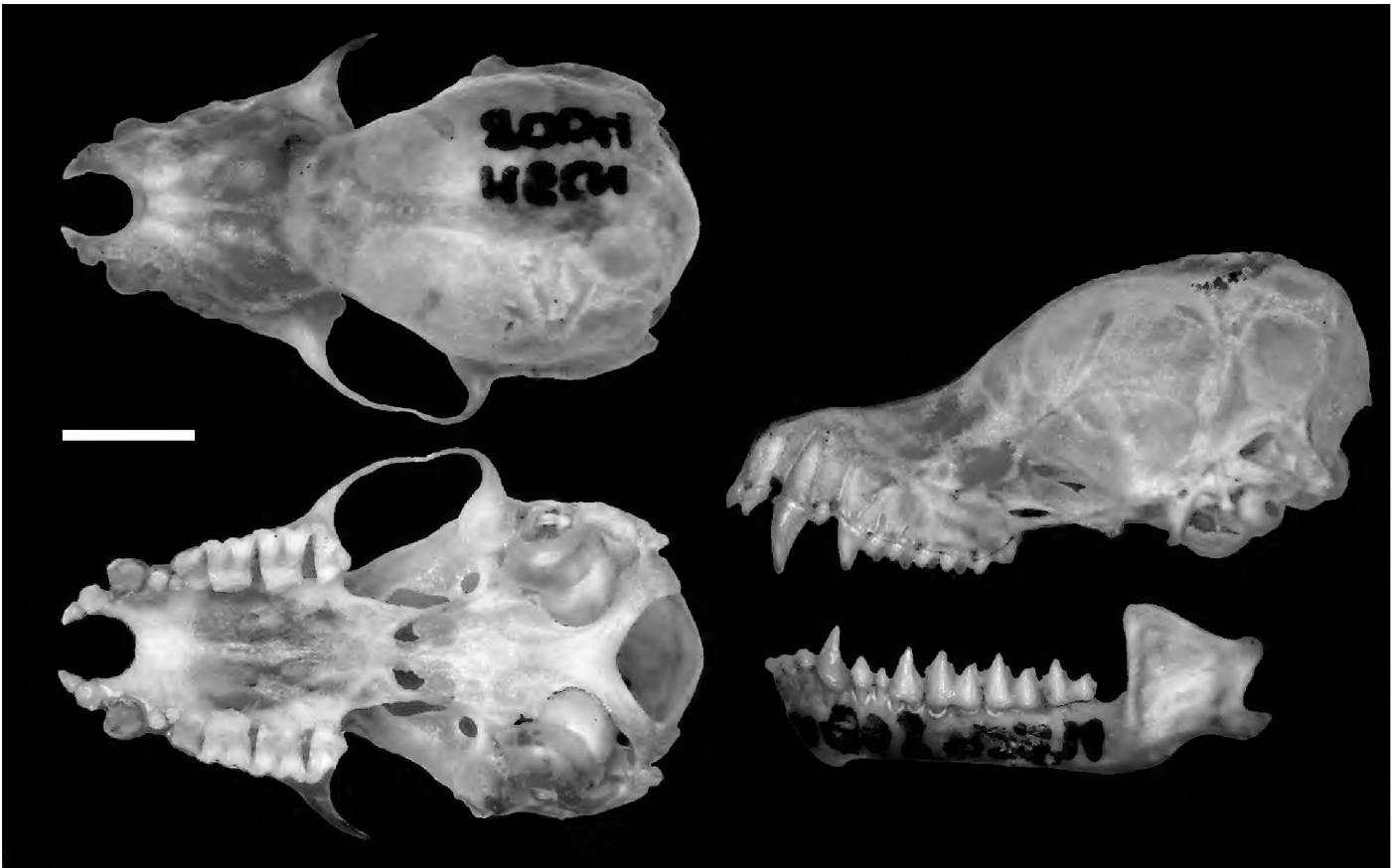
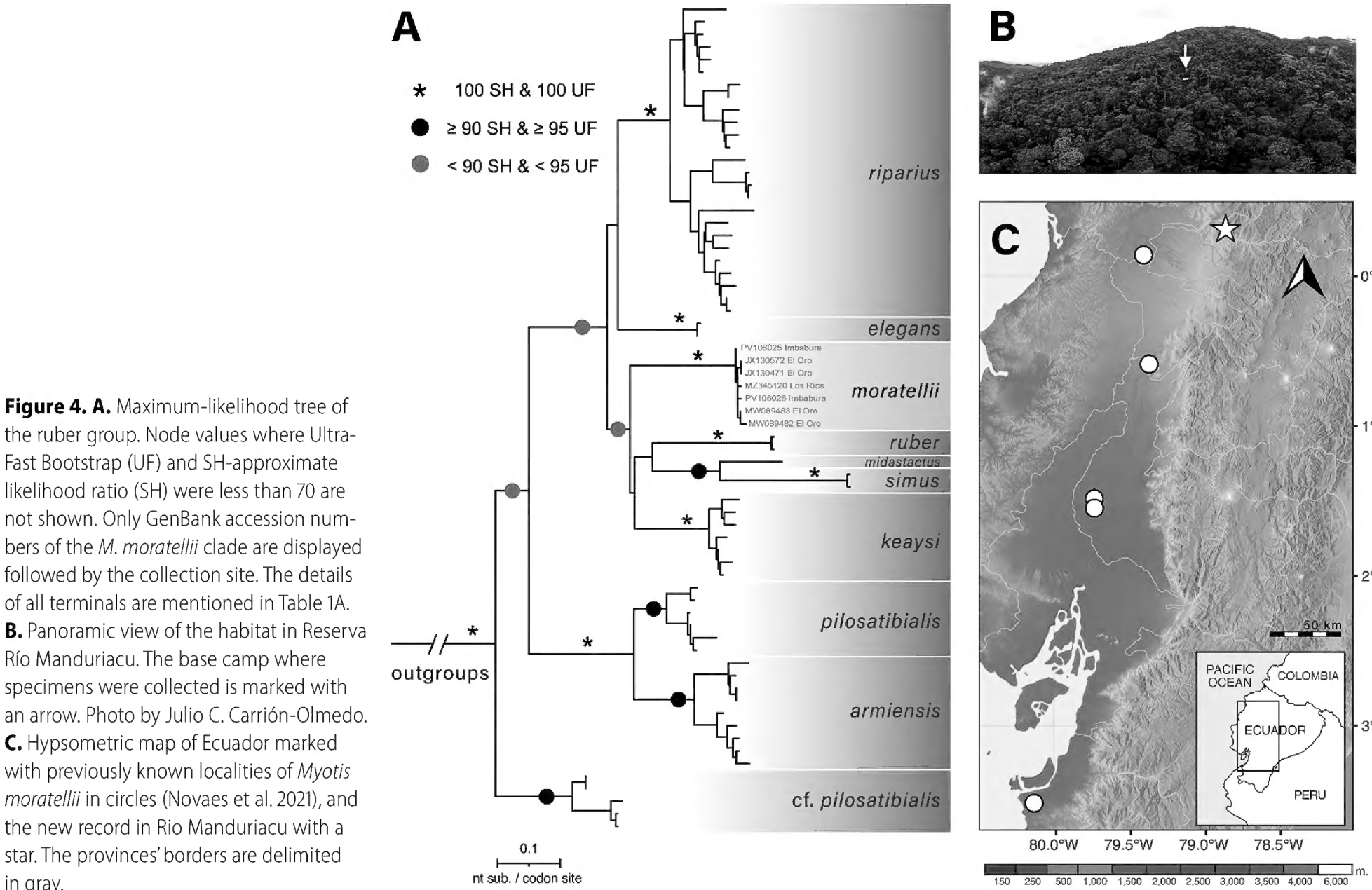


Table 1. Summary of measurement comparisons between the specimens collected in this study and *M. moratellii* representatives described in-text and Table 4 in Novaes et al. (2021). Asterisks highlight larger measurements in the Río Manduriacu specimens. Cranial measurements were taken in MECN 8004.

Measurements (in mm)	This study (<i>n</i> = 2)	Novaes et al. 2021 (<i>n</i> = 5–6)
Total length	77/81.5	76–89
Forearm length*	40/40.2	36.4 (35.0–38.2)
Tail length	31/31.5	32–38
Ear length	11.5/12.5	12.0 (11.5–13.0)
Length of dorsal fur	6/7	6.5 (5.0–7.5)
Length of ventral fur*	5/6	4.5 (4.0–5.5)
Body weight in grams	5.5/7.5	5–7
Greatest length of skull	13.7	13.89 (13.79–14.05)
Condyllo-canine length*	12.7	12.35 (12.18–12.55)
Condyllo-basal length	13.2	13.02 (12.82–13.28)
Condyllo-incisive length	13.9	13.89 (13.79–14.05)
Basal length*	12.4	11.89 (11.71–12.15)
Zygomatic breadth	—	9.04 (8.99–9.14)
Mastoid breadth	7.2	7.27 (7.15–7.36)
Braincase breadth	6.5	6.61 (4.81–6.48)
Postorbital breadth	3.5	3.55 (3.49–3.63)
Interorbital breadth	3.7	4.77 (4.60–5.15)
Breadth across canines*	4.0	3.86 (3.78–3.90)
Breadth across molars*	5.9	5.63 (5.51–5.79)
Maxillary tooththrow length*	5.4	5.25 (5.16–5.36)
Molariform tooththrow length*	3.4	3.04 (2.96–3.12)
Mandibular length*	10.9	10.22 (10.06–10.42)
Mandibular tooththrow length	5.6	5.63 (5.54–5.70)

UF = 100 and SH = 100). The *M. moratellii* clade was recovered as a sister lineage to *M. ruber* + *M. midastactus* + *M. simus* + *M. keaysi* with medium support (Figure 4A; UF = 88.9 and SH = 89). Lastly, the genetic distance of the Río Manduriacu specimens (PV106025, PV106026) to their conspecifics was 0.4%, whereas interspecific divergence values were greater than 11% (Table 2).

Natural history. The Reserva Río Manduriacu (Figure 4B) is a 9.66 km² protected area located in the Chocó ecoregion and the headwaters of the Río Manduriacu, in the western foothills of the Cordillera de los Andes (Albuja et al. 2012; Ron 2020), with altitudes ranging from 1,000 to 1,800 m a.s.l. The habitat is characterized by abundant palms and trees of the families Mimosaceae, Fabaceae, and Burseraceae. The forest canopy reaches 35 m or more, and the trees are covered by mosses, orchids, bromeliads, and ferns. Other bat species



found in sympatry with *M. moratellii* were *M. riparius* Handley, 1960, *Anoura cultrata* Handley, 1960, *Sturnira bogotensis* Shamel, 1927, *S. ludovici* Anthony, 1924, *S. koopmanhilli* McCarthy, Albuja & Alberico, 2006, *Platyrrhinus dorsalis* Thomas, 1900, and *Lophostoma occidentale* Davis & Carter, 1978.

DISCUSSION

The new records of *Myotis moratellii* in the Reserva Río Manduriacu (Imbabura Province), represent the tenth documented locality of the species in Ecuador and the first report in the foothill rainforest of the Chocó ecoregion, approximately 63 km away from the most northern site reported previously (Figure 4C). More importantly, it extends the altitudinal range from 150 to 1,240 m a.s.l., making it the highest altitudinal record for *M. moratellii* and with the greatest impact on the ecological knowledge of the species.

The specimens inhabiting this region have darker fur with Ochraceous-Orange and Maize Yellow patches of fur randomly dispersed through the body, in contrast to the lighter specimens reported by Novaes

Table 2. Genetic distances of ten species of the ruber group calculated with the Kimura 2-parameter model are shown as percentages. Within-group values are bolded and in the diagonal of the matrix. The asterisk marks specimens MECN 8004 and MECN 8096.

	1	2	3	4	5	6	7	8	9	10	11
1. <i>M. armiensis</i>	2.6										
2. <i>M. cf. pilosatibialis</i>	16.3	2.3									
3. <i>M. elegans</i>	19.6	16.2	0.2								
4. <i>M. keaysi</i>	20.4	19.5	11.9	1.9							
5. <i>M. midastactus</i>	21.8	22.6	13.8	14.2	—						
6. <i>M. moratellii</i> *	20.7	18.1	12.0	11.8	14.4	0.3					
7. <i>M. moratellii</i>	21.6	17.8	11.2	12.1	13.6	0.4	0.7				
8. <i>M. pilosatibialis</i>	8.7	15.3	18.4	20.1	22.5	16.1	16.3	2.0			
9. <i>M. riparius</i>	23.1	19.8	10.9	15.4	15.7	13.4	13.4	19.2	5.5		
10. <i>M. ruber</i>	25.7	20	15.6	14.4	14.8	14.5	14.0	21.4	16.9	0.3	
11. <i>M. simus</i>	23.1	23.6	16.5	17.1	10.3	18.1	17.3	22.3	17.5	18.5	0.3

et al. (2021), although the high support of the clade, and genetic distances less than 1%, suggest they are a single evolutionary lineage (Figure 4A, Table 2). The intraspecific differences of pelage color remain poorly explored and understood in bats (Davis and Castleberry 2010). Nevertheless, in the Neotropics, dark and light fur coloration in *Myotis lavalii* Moratelli et al., 2011 and *M. nigricans* Schinz, 1821 have been hypothesized to be associated with habitat differences (Moratelli and Wilson 2013).

In fact, the Reserva Río Manduriacu is characterized by a humid cloud forest (Figure 4B), while the specimens reported by Novaes et al. (2021) inhabit seasonal and broadleaf dry forests. Selective pressures in contrasting environments may, therefore, cause a shift in camouflage strategy leading to different coloration patterns (Caro 2005; Caro and Mallarino 2020). However, the numerous biological, ecological, and genetic factors causing these differences are still to be determined.

Finally, *M. moratellii* is known from a small number of museum specimens (Novaes et al. 2021; this study), which could lead us to consider it as a rare species. However, this perceived rarity, could be due to the lack of adequate field studies (Nivelo-Villavicencio et al. 2023) and the intricacy of its morphological identification. Knowledge of the natural history, ecology, and biogeography of the species is essential to design adequate conservation strategies (IUCN 2012), especially in an endangered and fragile ecosystem such as the Chocó (Roy et al. 2018). This note and other studies (Carrión-Bonilla et al. 2024; Korstian et al. 2024) exemplify and underscore the importance of increasing collections in poorly sampled geographic areas, sample sharing, collaborative efforts, and integrating new technologies and traditional approaches to untangle the yet to be discovered cryptic diversity within the genus.

ACKNOWLEDGEMENTS

We would like to thank the Bat Biology Foundation (BBF) who supported this research through the BBF fieldwork grant program, the Department of Biological Sciences at Texas Tech and Fundación Oso Andino for providing us with multiple sources of help to this project, either via funds or equipment. Thanks to the EcoMinga Foundation for their valuable effort to continue the conversation about the last threatened forests in Ecuador, especially Jimmy Alvarez, Lady Flores, and Dario Armas for their help during the field campaign. Julio Carrión-Olmedo is thanked for kindly providing us with a panoramic view of the Rio Manduriacu Reserve shown in Figure 4B. We are grateful to Dr. Mariano Sánchez who reviewed an early version of this manuscript. Finally, we thank Dr. Roberto Leonan M. Novaes, Dr. Carlos A. Carrión-Bonilla, and an anonymous reviewer who have improved the quality of this work.

ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

Funding

This study was partially financed and supported by the Bat Biology Foundation Fieldwork Grant, Paratus Sciences, Fundación EcoMinga, and Fundación Oso Andino.


Author contributions

Conceptualization: FXC, JB. Data curation: FXC, LS, JB. Formal analysis: FXC, JB. Funding acquisition: FXC, JB. Investigation: FXC, JB. Methodology: FXC, JB. Supervision: FXC, JB. Writing – original draft: FXC, JB. Writing – review and editing: FXC, LS, JB.

Author ORCID iDs

Francisco X. Castellanos  <https://orcid.org/0000-0003-0955-8185>

Laura Simba  <https://orcid.org/0009-0004-2442-7612>

Jorge Brito  <https://orcid.org/0000-0002-3410-6669>

Data availability

All data that supports the findings of this study are available in the main text and Appendix Table 1A.

REFERENCES

- Albuja L (1982) Murciélagos del Ecuador. First edition. Escuela Politécnica Nacional. Departamento de Ciencias Biológicas, Quito, Ecuador, 285 pp.
- Albuja L (1999) Murciélagos del Ecuador. Second edition. Escuela Politécnica Nacional. Departamento de Ciencias Biológicas, Quito, Ecuador, 288 pp.
- Albuja L, Mena VP (2004) Quirópteros de los bosques húmedos del occidente del Ecuador. *Politécnica* 25: 19–96.

- Albuja L, Almendáriz A, Barriga R, Montalvo LD, Cáceres F, Román JL (2012) Fauna de vertebrados del Ecuador. Instituto de Ciencias Biológicas, Escuela Politécnica Nacional, Quito, Ecuador, 490 pp.
- Baker RJ, Bradley RD (2006) Speciation in mammals and the genetic species concept. *Journal of Mammalogy* 87: 643–662. <https://doi.org/10.1644/06-mamm-f-038r2.1>
- Brito J, Koch C, Percequillo AR, Tinoco N, Weksler M, Pinto CM, Pardiñas UF (2020) A new genus of oryzomyine rodents (Cricetidae, Sigmodontinae) with three new species from montane cloud forests, western Andean cordillera of Colombia and Ecuador. *PeerJ* 8: e10247. <https://doi.org/10.7717/peerj.10247>
- Camacho MA, Menéndez-Guerrero PA, Horváth B, Cadar D, Murienne J (2024) A polytypic species revisited: phylogenetic and morphological variation, taxonomic status, and geographical distribution of *Trachops* (Chiroptera: Phyllostomidae). *Journal of Mammalogy* 105: 1001–1021. <https://doi.org/10.1093/jmammal/gyae067>
- Caro T (2005) The adaptive significance of coloration in mammals. *BioScience* 55: 125–136. [https://doi.org/10.1641/0006-3568\(2005\)055\[0125:tasoci\]2.0.co;2](https://doi.org/10.1641/0006-3568(2005)055[0125:tasoci]2.0.co;2)
- Caro T, Mallarino R (2020) Coloration in mammals. *Trends in Ecology & Evolution* 35: 357–366. <https://doi.org/10.1016/j.tree.2019.12.008>
- Carrión-Bonilla CA, Cook JA (2020) A new bat species of the genus *Myotis* with comments on the phylogenetic placement of *M. keaysi* and *M. pilosatibialis*. *Therya* 11: 508–532. <https://doi.org/10.12933/therya-20-999>
- Carrión-Bonilla CA, Ron S, Cook JA (2024) Species richness in *Myotis* (Chiroptera: Vespertilionidae): species delimitation and expanded geographic sampling reveal high Neotropical diversity. *Journal of Mammalogy* 105: 241–258. <https://doi.org/10.1093/jmammal/gyad124>
- Davis AK, Castleberry SB (2010) Pelage color of red bats *Lasiurus borealis* varies with body size: An image analysis of museum specimens. *Current Zoology* 56: 401–405. <https://doi.org/10.1093/czoolo/56.4.401>
- De Queiroz K (2007) Species concepts and species delimitation. *Systematic Biology* 56: 879–886. <https://doi.org/10.1080/10635150701701083>
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321. <https://doi.org/10.1093/sysbio/syq010>
- Hurtado N, Pacheco V (2017) Revision of *Neacomys spinosus* (Thomas, 1882) (Rodentia: Cricetidae) with emphasis on Peruvian populations and the description of a new species. *Zootaxa* 4242: 401–440. <https://doi.org/10.11646/zootaxa.4242.3>
- IUCN (2012) IUCN Red List categories and criteria: version 3.1. Second edition. IUCN, Gland, Switzerland and Cambridge, United Kingdom, 32 pp.
- Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. <https://doi.org/10.1038/nmeth.4285>
- Kimura M (1981) Estimation of evolutionary distances between homologous nucleotide sequences. *Proceedings of the National Academy of Sciences of the United States of America* 78: 454–458. <https://doi.org/10.1073/pnas.78.1.454>
- Korstian JM, Stevens RD, Lee TE, Baker RJ, Ray DA (2024) Unraveling the *Myotis* morass: ultraconserved-element analysis reveals introgression, cryptic diversity, and taxonomic trouble. *Journal of Mammalogy* 105: 259–276. <https://doi.org/10.1093/jmammal/gyad119>
- Kurta A, Kunz TH (1988) Capture methods and holding devices. *Ecological and behavioral methods for the study of bats* (T. H. Kunz ed.). Smithsonian Institution Press, Washington, DC, 1–30.
- Larsen RJ, Knapp MC, Genoways HH, Khan FAA, Larsen PA, Wilson DE, Baker RJ (2012) Genetic diversity of Neotropical *Myotis* (Chiroptera: Vespertilionidae) with an emphasis on South American species. *PLoS ONE* 7: 1–9. <https://doi.org/10.1371/journal.pone.0046578>
- Medellín RA (1993) Estructura y diversidad de una comunidad de murciélagos en el trópico húmedo mexicano. In: Medellín RA, Ceballos G (Eds.) *Avances en el estudio de los mamíferos de México Volumen 1*. Publicaciones Especiales Asociación Mexicana de Mastozoología, México DF, México, 333–354.
- Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30: 1188–1195. <https://doi.org/10.1093/molbev/mst024>
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37: 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Moratelli R, Wilson D (2011) A new species of *Myotis* Kaup, 1829 (Chiroptera, Vespertilionidae) from Ecuador. *Mammalian Biology* 76: 608–614. <https://doi.org/10.1016/j.mambio.2010.10.003>
- Moratelli R, Wilson D (2013) Distribution and natural history of *Myotis lavalii* (Chiroptera, Vespertilionidae). *Journal of Mammalogy* 94: 650–656. <https://doi.org/10.1644/12-mamm-a-257.1>
- Moratelli R, Wilson D (2015) A second record of *Myotis diminutus* (Chiroptera: Vespertilionidae): its bearing on the taxonomy of the species and discrimination from *M. nigricans*. *Proceedings of the Biological Society of Washington* 127: 533–542. <https://doi.org/10.2988/0006-324X-127.4.533>
- Moratelli R, Gardner AL, de Oliveira JA, Wilson DE (2013) Review of *Myotis* (Chiroptera, Vespertilionidae) from Northern South America, including description of a new species. *American Museum Novitates* 3780: 1–36. <https://doi.org/10.1206/3780.2>
- Moratelli R, Peracchi AL, Dias D, Oliveira JAD, De Oliveira JA (2011) Geographic variation in South American populations of *Myotis nigricans* (Schinz, 1821) (Chiroptera, Vespertilionidae), with the description of two new species. *Mammalian Biology* 76: 592–607. <https://doi.org/10.1016/j.mambio.2011.01.003>
- Muse SV, Gaut BS (2011) A likelihood approach for comparing synonymous and nonsynonymous nucleotide substitution rates, with application to the chloroplast genome. *Molecular Biology and Evolution* 11: 715–724. <https://doi.org/10.1093/oxfordjournals.molbev.a040152>
- Naser-Khdour S, Quang Minh B, Zhang W, Stone EA, Lanfear R (2019) The prevalence and impact of model violations in phylogenetic analysis. *Genome Biology and Evolution* 11: 3341–3352. <https://doi.org/10.1093/gbe/evz193>
- Nivelo-Villavicencio C, Brito J, Quezada AB, Alava L, Pardiñas UF (2023) Enlarging the knowledge on the Ecuadorean rodent *Rhagomys septentrionalis* (Cricetidae: Sigmodontinae) with remarks on rarity in sigmodontines. *Mammalia* 87: 367–373. <https://doi.org/10.1515/mammalia-2022-0080>

Novaes RLM, Cláudio VC, Wilson DE, Weber MM, Weksler M, Moratelli R (2024) Integrative taxonomy and evolutionary history reveal cryptic diversity in *Myotis riparius* (Chiroptera: Vespertilionidae). *Biological Journal of the Linnean Society* 142: 91–112. <https://doi.org/10.1093/biolinnean/blad123>

Novaes RLM, Cláudio VC, Carrión-Bonilla C, Abreu EF, Wilson DE, Maldonado JE, Weksler M (2022 [2021]) Variation in the *Myotis keaysi* complex (Chiroptera, Vespertilionidae), with description of a new species from Ecuador. *Journal of Mammalogy* 103: 540–559. <https://doi.org/10.1093/jmammal/gyab139>

Platt RN, Faircloth BC, Sullivan KAM, Kieran TJ, Glenn TC, Vandewege MW, Lee TE, Baker RJ, Stevens RD, Ray DA (2018) Conflicting evolutionary histories of the mitochondrial and nuclear genomes in New World *Myotis* bats. *Systematic Biology* 67: 236–249. <https://doi.org/10.1093/sysbio/syx070>

Ridgway R (1912) Color standards and color nomenclature. Published by the author. Biodiversity Heritage Library. <https://www.biodiversitylibrary.org/item/126819>. Accessed on: 2024-11-01

Ron SR (2020) Regiones naturales del Ecuador. Bioweb. Pontificia Universidad Católica del Ecuador. <https://bioweb.bio/faunaweb/amphibiaweb/RegionesNaturales>. Accessed on: 2024-12-18.

Roy BA, Zorrilla M, Endara L, Thomas DC, Vandegrift R, Rubenstein JM, Policha T, Ríos-Touma B, Read M (2018) New mining concessions could severely decrease biodiversity and ecosystem services in Ecuador. *Tropical Conservation Science* 11: 1940082918780427. <https://doi.org/10.1177/1940082918780427>

Ruedi M, Mayer F (2001) Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. *Molecular Phylogenetics and Evolution* 21: 436–448. <https://doi.org/10.1006/mpev.2001.1017>

Sahlin K, Lim MCW, Prost S (2021) NGSpeciesID: DNA barcode and amplicon consensus generation from long-read sequencing data. *Ecology and Evolution* 11:1392–1398. <https://doi.org/10.1002/ece3.7146>

Sanderson ND, Hopkins KM, Colpus M, Parker M, Lipworth S, Crook D, Stoesser N (2024) Evaluation of the accuracy of bacterial genome reconstruction with Oxford Nanopore R10. 4.1 long-read-only sequencing. *Microbial Genomics* 10: 001246. <https://doi.org/10.1099/mgen.0.001246>

Sikes RS (2016) 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy* 97: 663–688. <https://doi.org/10.1093/jmammal/gyw078>

Simmons NB, Cirranello AL (2025) Bat species of the world: a taxonomic and geographic database. Version 1.7. <https://batnames.org/>. Accessed on: 2025-02-10

Smith MF, Patton JL (1993) The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society* 50: 149–177. <https://doi.org/10.1111/j.1095-8312.1993.tb00924.x>

Stadelmann B, Lin LK, Kunz TH, Ruedi M (2007) Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. *Molecular Phylogenetics and Evolution* 43: 32–48. <https://doi.org/10.1016/j.ympev.2006.06.019>

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* 38: 3022–3027. <https://doi.org/doi:10.1093/molbev/msab120>

Tirira D (2017) A field guide to the mammals of Ecuador: including the Galapagos Islands and the Ecuadorian Antarctic zone. Asociación Ecuatoriana de Mastozoología y Editorial Murciélago Blanco. Publicación Especial sobre los mamíferos del Ecuador 11, Quito, Ecuador, 600 pp.

Tirira DG, Brito J, Burneo SF, Pinto CM, Salas JA, Comisión de Diversidad de la AEM (2024) Mamíferos del Ecuador: lista oficial actualizada de especies. Versión 2024.2. Asociación Ecuatoriana de Mastozoología, Quito, Ecuador. <http://aem.mamiferosdeecuador.com>. Accessed on: 2024-12-20

Yáñez-Fernández V, Marchán-Rivadeneira MR, Velazco PM, Burneo SF, Tinoco N, Camacho MA (2023) On the taxonomic identity of *Sturnira nana* Gardner and O’Neil, 1971 (Chiroptera: Phyllostomidae), from Ecuador, with the description of a new species of *Sturnira*. *American Museum Novitates* 2023: 1–28. <https://doi.org/10.1206/4001.1>

APPENDIX

Table 1A. *Myotis* specimens of the ruber group used in phylogenetics analyses. Species names correspond to the latest revision of Carrión-Bonilla and Cook (2020), Novaes et al. (2021), and Carrión-Bonilla et al. (2024). Acronyms of museums correspond to **ALP**: Federal Rural University of Rio de Janeiro, Seropédica, Brazil; **AMNH**: American Museum of Natural History; **CM**: Carnegie Museum of Natural History, Pittsburg, USA; **JAG**: Jane A. Groen; **MECN**: División de Mastozoología, Instituto Nacional de Biodiversidad, Quito, Ecuador; **MSB**: Museum of Southwestern Biology, Division of Mammals, The University of New Mexico; **MVZ**: Museum of Vertebrate Zoology, Mammal Collection, University of California Berkeley; **QCAZ**: Museo de Zoología, División de Mamíferos, Pontificia Universidad Católica del Ecuador; **SM**: Sumiko Matsumura; **SMF**: Senckenberg Museum of Frankfurt, Mammalogy Collection; **SNOMNH**: Sam Noble Oklahoma Museum of Natural History; **TTU** and **TK**: Natural Science Research Laboratory Mammal Collection at Texas Tech University; **USNM**: Smithsonian National Museum of Natural History.

Species	GenBank #	Voucher #	Source
<i>M. armiensis</i>	JX130435	TK 22503	Larsen et al. 2012
<i>M. armiensis</i>	JX130514	TK 104288	Larsen et al. 2012
<i>M. armiensis</i>	MW025265	MSB MAMM 262089	Carrión-Bonilla and Cook 2020
<i>M. armiensis</i>	MW025266	MSB MAMM 262237	Carrión-Bonilla and Cook 2020
<i>M. armiensis</i>	MW025267	MSB MAMM 262788	Carrión-Bonilla and Cook 2020
<i>M. armiensis</i>	MW025268	MSB MAMM 262085	Carrión-Bonilla and Cook 2020
<i>M. armiensis</i>	MW025269	QCAZ M 17245	Carrión-Bonilla and Cook 2020
<i>M. armiensis</i>	MW025274	QCAZ M 12461	Carrión-Bonilla and Cook 2020
<i>M. armiensis</i>	MZ345121	USNM MAMM 370890	Novaes et al. 2021
<i>M. cf. pilosatibialis</i>	JX130449	TK 13526	Larsen et al. 2012
<i>M. cf. pilosatibialis</i>	JX130489	CM 55764	Larsen et al. 2012
<i>M. cf. pilosatibialis</i>	JX130525	JAG 286	Larsen et al. 2012

Species	GenBank #	Voucher #	Source
<i>M. cf. pilosatibialis</i>	MF143477	TK 13525	Platt et al. 2018
<i>M. cf. pilosatibialis</i>	MW025270	MVZ MAMM 226977	Carrión-Bonilla and Cook 2020
<i>M. elegans</i>	JX130479	TK 101723	Larsen et al. 2012
<i>M. elegans</i>	JX130480	TK 101479	Larsen et al. 2012
<i>M. keaysi</i>	JX130516	QCAZ 11380	Larsen et al. 2012
<i>M. keaysi</i>	JX130517	QCAZ 11383	Larsen et al. 2012
<i>M. keaysi</i>	MW025273	MSB 70381	Carrión-Bonilla and Cook 2020
<i>M. keaysi</i>	MW089478	QCAZ MAMM12039	Carrión-Bonilla et al. 2024
<i>M. keaysi</i>	MW089479	QCAZ MAMM12038	Carrión-Bonilla et al. 2024
<i>M. keaysi</i>	MW089480	SNOMNH MAMM23783	Carrión-Bonilla et al. 2024
<i>M. keaysi</i>	MW089481	AMNH MAMM268651	Carrión-Bonilla et al. 2024
<i>M. midastactus</i>	MW323450	USNM MAMM 584502	Novaes et al. 2021
<i>M. moratellii</i>	JX130571	TK 135095	Larsen et al. 2012
<i>M. moratellii</i>	JX130572	TK 135069	Larsen et al. 2012
<i>M. moratellii</i>	MW089482	QCAZ MAMM9159	Carrión-Bonilla et al. 2024
<i>M. moratellii</i>	MW089483	QCAZ MAMM9623	Carrión-Bonilla et al. 2024
<i>M. moratellii</i>	MZ345120	USNM MAMM 513482	Novaes et al. 2021
<i>M. moratellii</i>	PV106025	MECN 8004	This study
<i>M. moratellii</i>	PV106026	MECN 8096	This study
<i>M. pilosatibialis</i>	JX130518	TK 14521	Larsen et al. 2012
<i>M. pilosatibialis</i>	JX130519	TK 34869	Larsen et al. 2012
<i>M. pilosatibialis</i>	JX130526	TK 14520	Larsen et al. 2012
<i>M. pilosatibialis</i>	MW025271	MVZ MAMM 226976	Carrión-Bonilla and Cook 2020
<i>M. pilosatibialis</i>	MW025272	MVZ MAMM 226973	Carrión-Bonilla and Cook 2020
<i>M. pilosatibialis</i>	MW025275	MVZ 224798	Carrión-Bonilla and Cook 2020
<i>M. riparius</i>	AF376866	MVZ 185996	Ruedi and Mayer 2001
<i>M. riparius</i>	AM261891	No voucher	Stadelmann et al. 2007
<i>M. riparius</i>	JX130436	TK 22703	Larsen et al. 2012
<i>M. riparius</i>	JX130469	TK 104572	Larsen et al. 2012
<i>M. riparius</i>	JX130473	CM 68443	Larsen et al. 2012
<i>M. riparius</i>	JX130474	CM 78659	Larsen et al. 2012
<i>M. riparius</i>	JX130485	TK 63827	Larsen et al. 2012
<i>M. riparius</i>	JX130486	TK 56784	Larsen et al. 2012
<i>M. riparius</i>	JX130488	TK 121165	Larsen et al. 2012
<i>M. riparius</i>	JX130490	CM 78651	Larsen et al. 2012
<i>M. riparius</i>	JX130491	TK 63402	Larsen et al. 2012
<i>M. riparius</i>	JX130492	TK 135564	Larsen et al. 2012
<i>M. riparius</i>	JX130506	TK 104318	Larsen et al. 2012
<i>M. riparius</i>	JX130513	TK 104098	Larsen et al. 2012
<i>M. riparius</i>	JX130515	TK 104573	Larsen et al. 2012
<i>M. riparius</i>	MW089486	QCAZ MAMM9165	Carrión-Bonilla et al. 2024
<i>M. riparius</i>	MW089488	QCAZ MAMM13894	Carrión-Bonilla et al. 2024
<i>M. riparius</i>	MW089490	QCAZ MAMM15339	Carrión-Bonilla et al. 2024
<i>M. riparius</i>	MW089492	QCAZ MAMM13936	Carrión-Bonilla et al. 2024
<i>M. riparius</i>	MW089493	SNOMNH MAMM36220	Carrión-Bonilla et al. 2024
<i>M. riparius</i>	MW089494	AMNH MAMM261808	Carrión-Bonilla et al. 2024
<i>M. riparius</i>	MW089495	MSB MAMM70383	Carrión-Bonilla et al. 2024
<i>M. riparius</i>	MW323447	ALP 10926	Novaes et al. 2021
<i>M. riparius</i>	MW323448	ALP 10927	Novaes et al. 2021
<i>M. riparius</i>	MW323449	ALP 11024	Novaes et al. 2021
<i>M. ruber</i>	AF376867	MVZ 185999	Ruedi and Mayer 2001
<i>M. ruber</i>	MF143478	MVZ 185692	Platt et al. 2018
<i>M. simus</i>	JX130481	TTU 46348	Larsen et al. 2012
<i>M. simus</i>	MF143473	TK 22688	Platt et al. 2018
Outgroups			
<i>M. cf. nigricans</i>	JX130496	TK 64475	Larsen et al. 2012
<i>M. lavalii</i>	AF376864	MVZ-M 185682	Ruedi and Mayer 2001
<i>M. levis</i>	JX130475	TK 49150	Ruedi and Mayer 2001